

## Abstract

Mycorrhizal fungi form symbiotic relationships with plants and are essential to the development of an ecosystem. Urban environments can affect the quality of these ecosystems through their effects on soil quality and fungal biodiversity. Our project aims to use fungal samples from New York City parks to observe some of the biodiversity of the urban fungal community. We collected visible fungal samples from parks and used the Rapid DNA isolation protocol to extract DNA that would be sequenced using Sanger sequencing and analyzed using the multiple sequence alignment and phylogenetic tree building tools available on DNA Subway. We collected 10 fungal samples from Kissena and Prospect Parks, which appeared to be a variety of fungi and lichens. Some were pathogenic, one was non-native, and one was commonly found in urban areas, indicating a reasonable amount of biodiversity although in pathogenic species, which would imply that the ecosystem's health is less than ideal. These findings could be used to indicate a need for better management of the ecosystems in New York City Parks in terms of regulating pathogenic fungi and facilitating the growth of beneficial fungi.

## Introduction

Mycorrhizal fungi form symbiotic relationships with plants and facilitate the absorption of nutrients from the soil, enhancing plant growth (Veysi et al.). Soil that has the right conditions to support a large, diverse population of these fungi allows for better plant growth. These conditions can be negatively affected by proximity to urbanized areas, which notably increase the pH of the soil, degrading the fungus community (Ivashchenko et al.). Research has been done about the communities of mycorrhizal fungi in green roofs and parks around New York City and suggested that fungal communities were significantly compositionally clustered and that they were very important parts of these ecosystems (Discenza et al.). Our project aims to look specifically at the biodiversity of the mycorrhizal fungus communities within the New York City area, which is a good indicator of the health of the plant life and larger ecosystems that depend on it.

We intended to use soil samples to detect mycorrhizal environmental DNA from urban NYC. Due to limitations imposed by Covid-19, we were unable to do so, and instead collected samples of visible fungi and lichen from parks, including Kissena Park. We expected to find DNA from various species of plants and fungi, including native species such as *Sclerocystis dussii* and *Glomus pubescens*, as documented by the New York Botanical Gardens. We compared the sequences to identify trends within isolated populations from different sample sites including certain mutations unique to specific sites. We compared our data to existing genomic data from existing research in other locations to identify mutations and trends specific to the fungus population of the city. We also used the comparison to analyze the biodiversity present within the individual fungal communities and within the larger city population.

## Acknowledgements

We would like to thank the DNA Learning Center for running this program even through covid, and our mentors, Craig Trester and Sigrid Jakob, for their enormous help and guidance in executing this project.

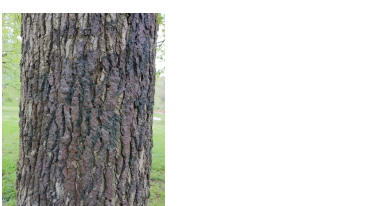


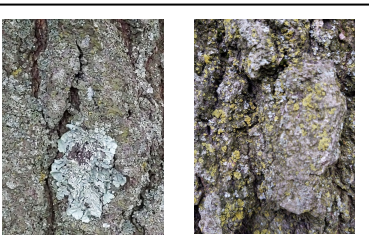

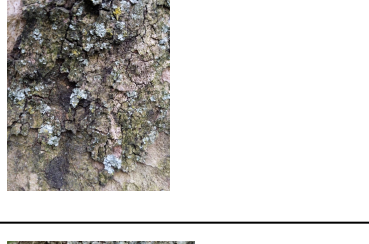
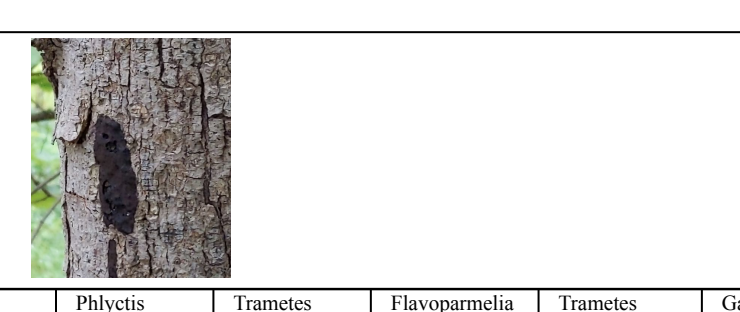

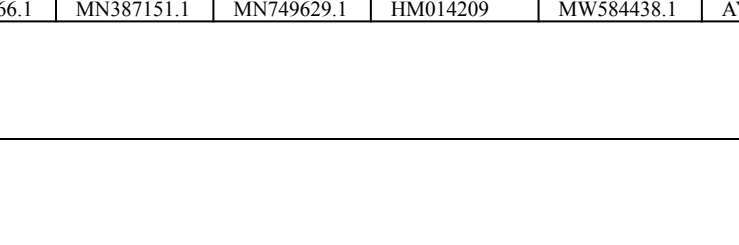



## Materials & Methods

We collected a total of 10 samples from parks throughout the city, including Kissena and Prospect Parks, within a limited time frame from April 26 to May 8 to prevent variability of the fungi available based on the time the sample was taken. We collected data about the surroundings of the samples as well and tried to collect samples that were near or on trees. We then processed these samples and isolated DNA using the Rapid DNA isolation protocol. We had to reuse pestles when grinding the samples in the DNA isolation protocol, so in order to sterilize them, we rinsed them out with tap water and then soaked them in rubbing alcohol for 5 minutes before allowing them to air dry and be reused. We amplified for the ITS1 region using the ITS1-F and ITS2 primer pair, as used in the paper by McGuire et al. We used Sanger sequencing to produce a barcode that we could compare to existing data from DNA Subway, Kbase, and GenBank. We compared our barcodes to each other using DNA Subway and used all three databases to get the largest number of additional samples with which to compare our populations.







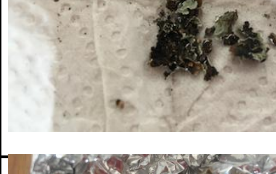

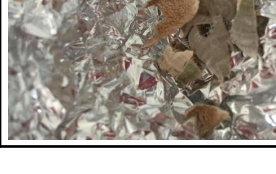

We used the tools available on DNA Subway, including multiple sequence alignment and phylogenetic tree building, to compare the presence, absence, and relative abundance of the various sample and existing sequences over the various locations. We analyzed the diversity using the number of different sequences present in our data, and also determined whether each geographic location contains a variety of sequences or the variety is present only in the larger geographic range. We also determine certain mutations that were unique to our sample sites, showing up commonly within them but not outside them. We used the frequency of mutations looked for across the various locations to determine the genetic diversity and trends that they represent.

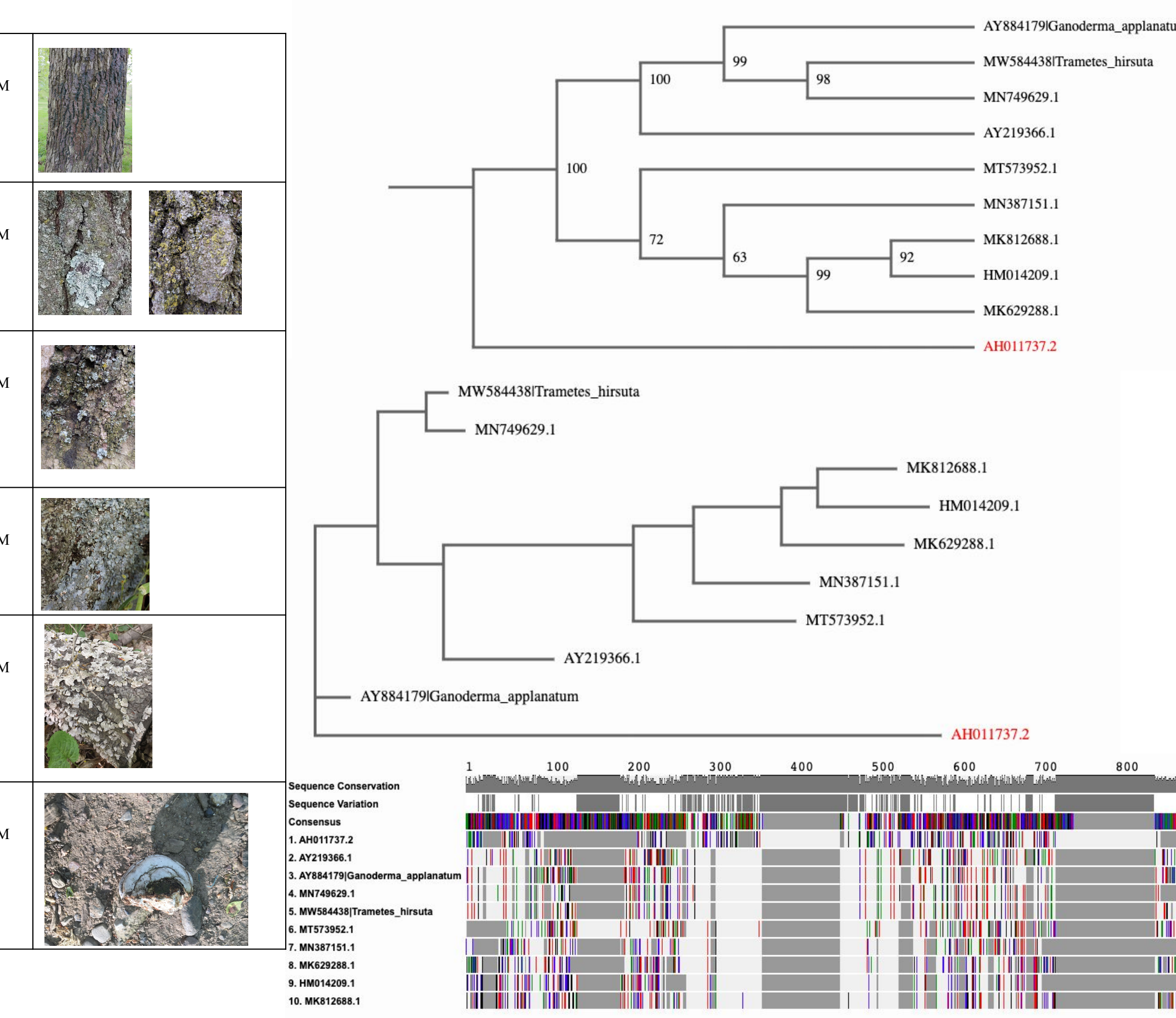
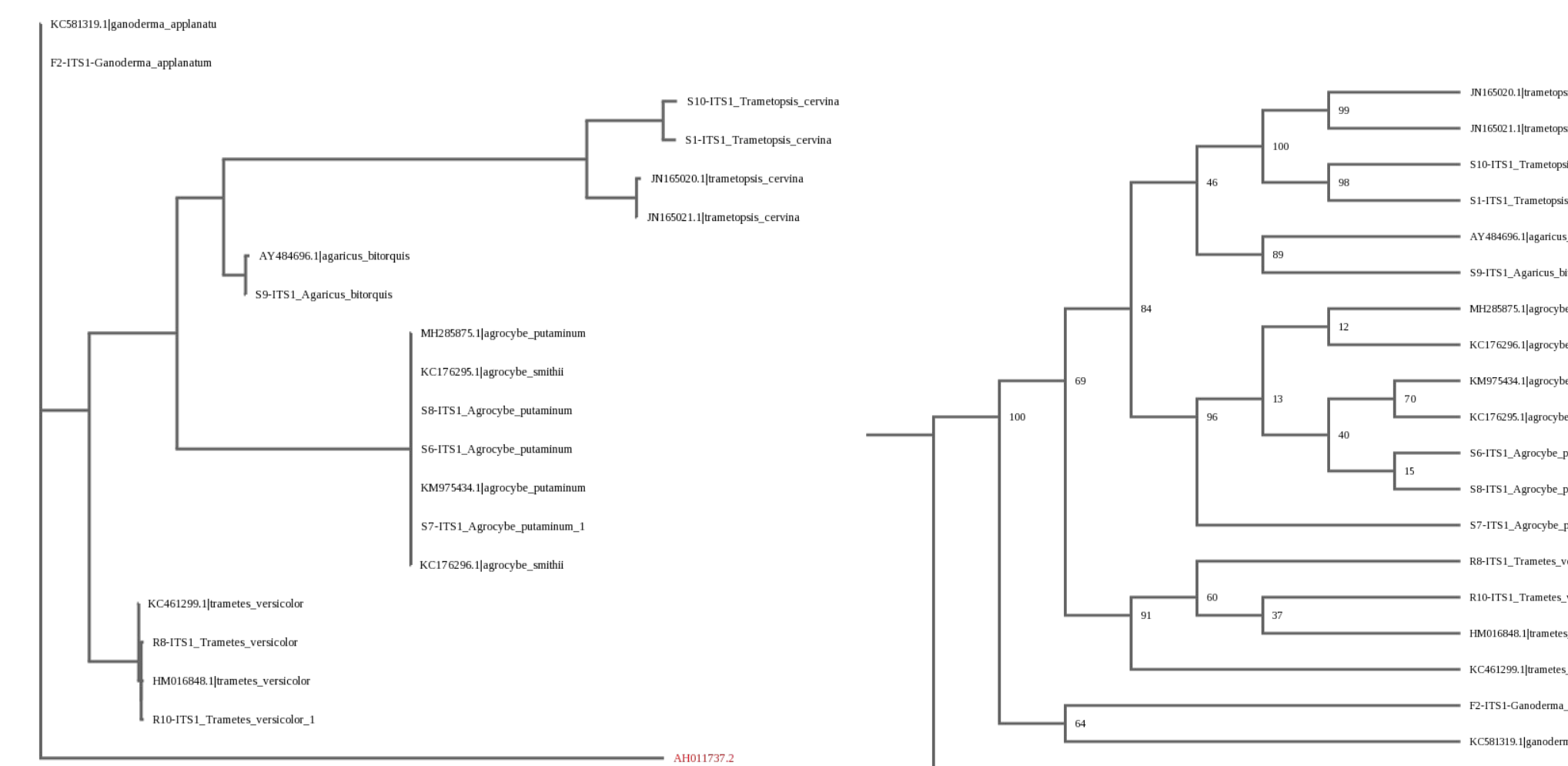
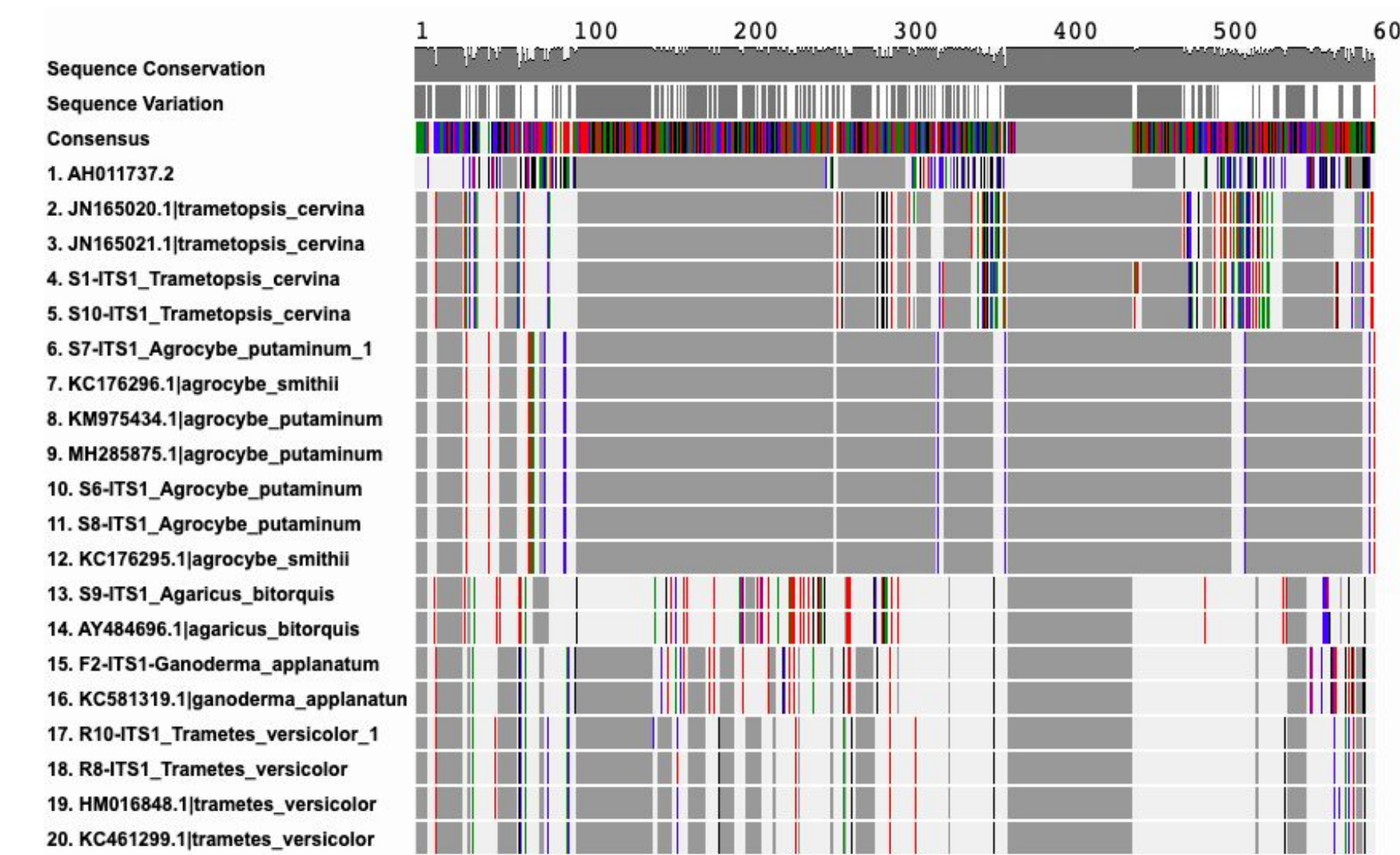
## Results

We found a total of 10 samples throughout Kissena and Prospect Parks. We have compiled tentative phylogenetic trees based on sequences obtained from GenBank of the ITS1 and ITS2 regions of the species collected as identified by iNaturalist. Below are the species identified by iNaturalist and their corresponding accession number and version from GenBank, which is how they are identified on the phylogenetic trees, as well as one neighbor-joined and one maximum-likelihood phylogenetic tree derived from the GenBank sequences. There is also the multiple-sequence alignment. Pending more data, we were also able to get sequences from 9 of the samples of 36 collected throughout the city.

Number	Tentative ID	Location	Location Description	Observed	Photos	D5	Unknown	Kissena Park	Found on a tree, maybe Liquidambar styraciflua, near a path.	May 8, 2021	4:07 PM UTC	
D1	Phlyctis argena	Kissena Park, East Flushing - Queens, NY, USA	Found on bark on ground near tree, possibly Acer platanoides.	Apr 26, 2021	6:02 PM EDT		D5					
D2	Trametes conchifer	Kissena Park, East Flushing - Queens, NY, USA	On a tree trunk and roots, probably Salix babingtonia. Near a moderately traveled path.	Apr 26, 2021	5:24 PM EDT		D6	Flavoparmelia caperata	Found near a path, on a tree, maybe Quercus shumardii.	May 8, 2021	4:18 PM UTC	
D3	Phlebia radiata	Kissena Park, East Flushing - Queens, NY, USA	On tree trunk and roots, probably Salix babingtonia. Near moderately traveled path. Also present with Trametes conchifer.	Apr 26, 2021	5:33 PM EDT		D7	Melanetia subaurifera	Near path. On tree, maybe Platonicus occidentalis.	May 8, 2021	4:38 PM UTC	
D4	Kretzschmaria deusta	Kissena Park	Out of the way, on a tree, maybe Acer saccharum.	May 8, 2021	3:53 PM UTC		D8	Lepraria finkii	Near a path, on a tree, maybe Quercus palustris.	May 8, 2021	4:58 PM UTC	
							D9	Trametes hirsuta	Growing on a dead fallen down tree trunk.	May 2, 2021	5:27 PM EDT	
							D10	Ganoderma applanatum	Large, growing on a tree.	May 2, 2021	5:30 PM EDT	

## Tables & Figures

Proposed Name	Photo	Plateau sp.	
Camillea asp.		Trametes cinnabarinus	
Camillea tinctor		Trichaptum bifforme	
Candelaria concolor		Xanthoparmelia	
Calendalaria concolor		Trichaptum bifforme	
Physcia			



## Discussion

Our proposal aimed to extract eDNA from soil samples to determine biodiversity, but due to Covid-19 restrictions, we were unable to complete the wet lab procedures necessary to work with those samples, and instead took fungal samples from parks. We found a variety of species in a variety of locations, indicating the presence of a reasonable amount of fungal species in the park ecosystems. Given the tentative phylogenetic trees, we can assume a reasonable level of biodiversity among the fungal population. Some of the fungal species would be considered parasitic or invasive, however, indicating that the actual health of the ecosystem is less than ideal.

*Kretzschmaria deusta* is a pathogenic fungus that causes a soft rot in the lower stem or root of trees in the temperate Northern Hemisphere ("Brittle Cinder (Kretzschmaria deusta)"). *Lepraria finkii* is a common type of lichen in North America north of Mexico. It is remarkably able to withstand pollution and disturbance, and therefore survives well in urban environments (Lendemer, James). *Melanetia subaurifera* is a lichen native to Canada with seasonal spikes in May and August ("Abraded Camouflage Lichen (Melanetia Subaurifera)"). *Phlebia radiata* is a common species of crust fungus in the Northern Hemisphere. It causes a white rot in fallen logs and branches of various kinds of trees ("Wrinkled Crust (Phlebia Radiata)"). *Phlyctis argena* is a lichen native to Canada and the US. It has a seasonal spike in January and possibly April ("Whitewash Lichen (Phlyctis Argena)"). *Trametes conchifer* is found in North America on trees ("Little Nest Polypore (Trametes Conchifer)"). *Flavoparmelia caperata* is a common lichen that grows on tree bark and occasionally rocks in a variety of regions, the US included. It has seasonal spikes in February, April, and September, but is relatively common year-round ("Common Greenshield Lichen (Flavoparmelia caperata)"). *Trametes hirsuta* is a pathogenic fungus that grows on the dead wood of deciduous trees. It is not native to the US ("Hairy Bracket (Trametes Hirsuta)"). *Ganoderma applanatum* is a parasitic fungal species native to the US that grows on living and dead wood. It has slight spikes around April and August, but is relatively common year-round ("Artist's Bracket (Ganoderma Applanatum)").

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